

## REMARKS

Claims 1 to 15 and 20 are in the case.

With this amendment, Applicant respectfully requests permission to amend the drawings to overcome the informality noted by the Examiner - i.e. that the individual drawings were not labeled. Applicant has also removed some of the written material therein.

The disclosure has been amended to put in the titles requested by the Examiner. In so doing, certain corrections have been made to the description of the figures since errors were present in the original description.

Applicant has also revised the disclosure to clearly indicate which figures are referred to in any particular instance and particularly where there are multiple figures.

In the claims, Applicant has amended Claim 4 to clearly define the meaning of the term "x" in the formulas. Furthermore, Claims 5, 9 and 10 have been amended to substitute the word "hectare" for the abbreviation "ha".

In Claim 20, the typographical error has been corrected.

Reconsideration of the Examiner's rejection of the claims under 35 U.S.C. 103 is respectfully requested in view of the following comments.

As noted by the Examiner, the claims are drawn to a method of controlling soilborne pathogens by adding to the soil a nitrogen containing material and a pH reducing agent to reduce the soil pH to below 5.5.

Anderson (6,074,638) does point out that potato scab is controlled by reducing soil pH as is well known in the art. The mechanism by which potato scab disease is

controlled by acidity is unknown, except that it has been established for decades that it cannot be cultured in the laboratory under extreme acid conditions. However, reducing the soil pH has only a temporary effect on controlling potato scab disease since once the pH returns to normal levels (as it will) the disease reoccurs because the pathogen is not killed.

In the present invention, soil pH is reduced to convert nitrite to nitrous acid. Pathogens are then killed by toxic nitrous acid. Evidently, the disease will not reoccur since the pathogen is not present to produce the disease. Thus, the method is substantially different from what is shown in the art. As a side note, there are acid tolerant strains of *S. scabies* which reduced pH does not control.

The Raskin et al patent relates to a method for removing metals from soil by acidifying the soil to make the metal soluble and take it up by plant roots. This is completely removed from the goal and method of the present application and is not believed to be pertinent. As mentioned above, the present invention has particular nitrogen compounds to soil in conjunction with acidifying the soil to convert nitrate to nitrous acid for the purpose of killing the pathogens. There is no effect of increasing the availability of nutrients but rather degeneration of a toxin to pathogens. Nitrous oxide evidently is not a nutrient.

Weltzien et al describes a means of producing a fertilizer from ascorbic acid and algae and humic acid to supply nutrients to plants. The present invention uses acid generated compounds not to reduce a fertilizer or increase nutrient uptake by plants, but rather is a method to generate a toxic compound.

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The patent to Babel Jr. is similar to that of Anderson et al - increasing the availability of metal for uptake by plants. In this invention, citric acid is used both to increase the solubility of iron (solubilized iron - minerals to produce free iron) and to Chelete Iron (mind free iron) make it more available as a nutrient for plants. Again, this is far removed from the teachings of the present invention.

As noted above, the nitrogen utilized by Applicant is not a nutrient source, but rather is present to produce a pathogen killing toxin.

In view of the above, it is now believed this application is in order for allowance, and such action is respectfully requested.

Respectfully,




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Barbara Duffus



**Version with markings showing the changes made**

Paragraphs 3 and 5 through 9 on page 4.

Figures 1a to [1k] 1j are graphs indicating soil pH and the number of microsclerotia germinated as well as  $\text{NH}_3$  concentration in soil,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  content in two different soils;

[Figures 3a through 3h are graphs showing the effect with and without the nitrification inhibitor DCD;] Figures 3a, 3b and 3c are graphs showing microsclerotia germinated in a soil amended with various amounts of urea;

[Figures 4a through 4d are graphs showing the number of microsclerotia germinated and  $\text{HNO}_2$  concentration;] Figures 4a through 4h are graphs showing the effect with and without the nitrification inhibitor DCD;

[Figures 5a through 5m are graphs similar to those in Figure 3 in a different type of soil;] Figures 5a through 5d are graphs showing the number of microsclerotia germinated and  $\text{HNO}_2$  concentration;

[Figure 6 is a graph illustrating the number of microsclerotia germinated after being exposed for two weeks to various concentrations of  $\text{NH}_3$ ;] Figures 6a to 6f are graphs showing microsclerotia germinated, soil pH and  $\text{HNO}_2$  concentration of a soil amended with various amounts of  $(\text{NH}_4)_2\text{SO}_4$ , with and without a nitrification inhibitor;

Figure 7 is a graph illustrating the number of microsclerotia germinated [for various time counts after exposure] after being exposed for two weeks to various concentrations of  $\text{NH}_3$ ;



**Version with markings showing the changes made**

Paragraphs 1 through 6 on page 5.

Figure 8 is a graph illustrating the number of microsclerotia germinated [at various times after being exposed to various concentrations of  $\text{HNO}_2$  and a citric acid buffer;] for various time counts after exposure to various concentrations of  $\text{NH}_3$ ;

Figure 9 is a graph illustrating the number of microsclerotia germinated at various times after [exposure] being exposed to various concentrations of [30 mL]  ~~$\text{HNO}_2$  and a citric acid buffer;~~

Figure 10 is a graph illustrating [the peak concentration of  $\text{NH}_3$  for a soil amended with 2% MBM;] the number of microsclerotia germinated after exposure to various concentrations of 30 mL  $\text{HNO}_2$  and a citric acid buffer;

Figure 11 is a graph illustrating [soil pH in response to  $\text{H}_2\text{SO}_4$ ;] the peak concentration of  $\text{NH}_3$  for a soil amended with 2% MBM;

[Figures 12a through 12k are graphs illustrating the number of microsclerotia germinated, soil pH,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  content and  $\text{HNO}_2$  concentration in a soil solution and two different soils;] Figure 12 is a graph illustrating soil pH in response to  $\text{H}_2\text{SO}_4$ ;

Figures 13a through 13j are graphs illustrating the number of microsclerotia germinated, soil pH,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  content and  $\text{HNO}_2$  concentration in a soil solution and two different soils;

[Figures 14a, 14b, and 14c are graphs illustrating the germination of microsclerotia and percent colony forming units of different organisms.] Figures 14a and 14b show the germination of microsclerotia after submergence in a citric acid buffered solution at differing pHs;

### **Version with markings showing the changes made**

Figures 15a to 15f show percent colony forming units of different types of spores after submergence in citric acid buffered solution containing various levels of  $\text{HNO}_2$ .

Paragraph 3 on page 6.

Ammonia in excess of  $65 \text{ mg N kg}^{-1}$  soil ( $20 \text{ mM NH}_3$ ) coincided with a rapid loss in the viability of microsclerotia [(Fig. 1)] **(Fig. 1a to 1j)**. In two experiments MBM or soya meal (SM) were added to various concentrations (0, 0.25, 0.5, 1, and 2% weight/weight) to soils from two locations namely, Beauseart and Thorndale. Quite high levels of ammonia accumulated in the Beauseart soil amended to 2%, but none was detected in the Thorndale soil. The viability of microsclerotia remained above 60% in Thorndale soil compared to less than 10% in Beauseart soil amended to 2% (weight/weight). When 1% MBM or SM was added to Beauseart soil a gradual decline in

Paragraph 2 on page 7.

The Thorndale soil amended to 2% MBM or SM failed to accumulate sufficient  $\text{NH}_3$  to kill microsclerotia. This provided the opportunity to confirm  $\text{NH}_3$  as responsible for killing of microsclerotia by inducing high levels of  $\text{NH}_3$  in the Thorndale soil by determining the survival of microsclerotia. This approach consisted of adding high rates of MBM to the Thorndale soil. Thus MBM was applied at the rates of 0.2 and 4% (weight/weight). The 2% amendment resulted in negligible  $\text{NH}_3$  accumulation and survival of microsclerotia greater than 50% by the end of the study [(Fig. 2)] **(Fig. 2a to 2e)**. In contrast at 4% MBM,  $\text{NH}_3$  accumulated to above

## Version with markings showing the changes made

Paragraph 2 on page 7 (Cont'd)

150 mM one week following amendment and continued to the end of the study. This corresponded to complete death of microsclerotia.

Paragraph 2 on page 19.

An example of the importance of nitrification rate in producing  $\text{HNO}_2$  is evident in a study in which 400 or 800 mg N  $\text{kg}^{-1}$  as  $(\text{NH}_4)_2\text{SO}_4$  was added to Beauseart and Mackenzie soils. The Beauseart soil was air-dried and stored for 1.5 years prior to initiation of the experiment. The Mackenzie soil was recently collected and stored at  $4^\circ\text{C}$  and at field moisture content. Recently collected Beauseart soil was shown previously to generate  $\text{HNO}_2$  in response to  $(\text{NH}_4)_2\text{SO}_4$  addition [(Fig. 5)] (Fig. 5a to 5d). However, the air-dried Beauseart soil failed to accumulate  $\text{HNO}_2$  and kill microsclerotia [(Fig. 13)] (Fig. 13a to 13j). In comparison, the Mackenzie soil has rapid nitrification, associated reduction in soil pH, accumulation of  $\text{HNO}_2$ , and death of microsclerotia. The population of autotrophic nitrifying bacteria at the start of the experiment was higher in the Mackenzie soil ( $1.1 \times 10^5 \text{ g}^{-1}$  soil) compared to the Beauseart soil ( $5.8 \times 10^3 \text{ g}^{-1}$  soil) likely accounting for differences in nitrification rate between soils.

**Version with markings showing the changes made**

**Claim 4 (Once amended)**

The method of claim 2 wherein said nitrogen containing material is selected from a group consisting of animal manures, sewage sludge, animal by-products, chitinaceous materials, oil- seed materials, urea,  $\text{NH}_4\text{x}$  and  $\text{xNO}_2$  [compounds.] compounds wherein x is selected from the group consisting of salts of ammonium and salts of nitrite.

**Claim 5 (Once amended)**

The method of claim 2 wherein said nitrogen containing material is added at a rate of between 200 kg [N/ha] N/hectare and 1000 kg [N/ha] N/hectare.

**Claim 9 (Once amended)**

The method of claim 5 wherein said nitrogen containing material is applied at a rate of between 400 kg [N/ha] N/hectare and 800 kg [N/ha] N/hectare.

**Claim 10 (Once amended)**

The method of claim 7 wherein said nitrogen containing material is applied at a rate of between 600 kg [N/ha] N/hectare and 1000 kg [N/ha] N/hectare.

**Claim 20 (Once amended)**

The method of Claim 1 further including the step of measuring the pH of the soil, measuring the buffering capacity of said soil, and adding said nitrogen containing material and said pH reducing agent to said soil when said buffering capacity is below 2 uL  $\text{H}_2\text{SO}_4$  [g/soil] /g soil.